The Veliger 50(3):185–189 (October 1, 2008)

THE VELIGER

Galba truncatula Müller, 1774 (Pulmonata: Lymnaeidae) in Argentina: Presence and Natural Infection by *Fasciola hepatica* (Linnaeus, 1758) (Trematoda: Digenea)

LAURA ISSIA,[1] SILVIA M. PIETROKOVSKY,[1] FLORENCIA KLEIMAN,[1] PABLO CARMANCHAHI[2] AND CRISTINA WISNIVESKY-COLLI[1]

[1] Unidad de Ecología de Reservorios y Vectores de Parásitos, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pab. II, C1428EHA, Buenos Aires, Argentina
(e-mail: Laura Issia: issia@ege.fcen.uba.ar)
[2] Asentamiento Universitario de San Martín de los Andes, Universidad Nacional del Comahue, Neuquén, Argentina

Abstract. We report the finding of *Galba truncatula* in Argentina. In June and August 2006, 157 snails were collected from a stream in Sierras del Palauco, Province of Mendoza, northern Patagonia, Argentina. *Fasciola hepatica* infection was detected in one of 50 specimens collected in June. In the Americas, this snail was reported from Bolivia and Chile, and its occurrence in Argentina may reflect an ongoing process of geographic expansion. In Argentine Patagonia, *Lymnaea viatrix* has been regarded as the only lymnaeid involved in the transmission of *F. hepatica*, but our results suggest that *G. truncatula* may also be playing a role.

INTRODUCTION

Mollusks are used as first intermediate hosts by all species of digenetic trematodes (Esch et al., 2002). In particular, the cosmopolitan snails of the family Lymnaeidae are involved in the transmission of some digeneans of veterinary and medical importance, such as *Fasciola hepatica* (Linnaeus, 1758), the causative agent of fasciolosis (Malek, 1985).

The endemicity of fasciolosis in a region depends on the presence of intermediate hosts adapted to particular environmental conditions, and therefore the identification of local lymnaeid species is essential for the design of effective control strategies. In the native fauna of Argentina, species of Lymnaeidae so far reported are *Lymnaea diaphana* King, 1830; *Lymnaea pictonica* Rochebrune & Mabille, 1885; *Lymnaea viatrix* Orbigny, 1835 (Hubendick, 1951; Paraense, 1976, 1982; Malek, 1985; Kleiman et al., 2004); and *Pseudosuccinea columella* Say, 1817 (Paraense, 1982; Prepelitchi et al., 2003). At present, the only species incriminated in the transmission of *F. hepatica* are *P. columella* (Prepelitchi et al., 2003) and *L. viatrix*, which shows the broadest distribution (Rubel et al., 2005; Cucher et al., 2006; Kleiman et al., 2007).

In this work, we report *Galba truncatula* Müller, 1774 in Argentina, and provide evidence of natural infection of this snail with *F. hepatica*.

MATERIAL AND METHODS

The study was performed in a stream at an altitude of 1943 m, located in Sierras del Palauco (35°57′S 69°24′W), Department of Malargüe, Province of Mendoza, northern Patagonia, Argentina (Figure 1). The climate in the study area is cold and arid, with a mean annual temperature of 21.3°C (mean temperature of the coldest month, July: −2.3°C; mean temperature of the warmest month, January: 27.9°C). Winter is the rainy season, and the mean annual precipitation is 198 mm. Biogeographically, the region is included within the Andean–Patagonian domain, Patagonian province, Payunia district, which is characterized by xerophytic vegetation (Cabrera & Willink, 1980). The human population is small and houses are scattered over a large area (density: <1 inhabitant per km^2). The economic activity is mainly based on the rearing of goats, followed by sheep and cattle. Local inhabitants have mentioned the presence of *F. hepatica* in their livestock.

Two surveys were performed, one in June and one in August 2006 (winter). In the studied stream, 157 snails were hand-collected between 12:00 pm and 4:00 pm. Water temperature and pH were recorded simultaneously. Samples of aquatic vegetation associated with the snails were taken for further identification. Snails were transported alive to the laboratory in plastic flasks

Figure 1. Map of Argentina (sparse dots). The asterisk indicates the studied area, Sierras del Palauco, in the Province of Mendoza (dense dots).

containing water and vegetation from the collecting site.

The taxonomic determination of snails was performed in 33 of the largest specimens collected from both surveys, so as to increase the probability of sexual maturity. These were relaxed, sacrificed, preserved in Railliet-Henry's fluid (Paraense, 1984), and taxonomically determined by features of the shell and the male reproductive system. Shell measures recorded from these specimens were as follows: length from the apex to the anterior margin (shell length, SL); maximum shell width (SW); aperture length (AL); and aperture width (AW). The following ratios were calculated: SL/SW, SL/AL, and penis sheath length/prepuce length. The shell length was measured in the 124 remaining snails. All measurements were made using a stereoscopic microscope with a graduated eyepiece.

Live snails were individually placed in small containers with dechlorinated tap water and then exposed to light to stimulate cercariae shedding. Preserved and live snails were dissected to detect trematode larvae in viscera. Determination of *F. hepatica* infection was based on the morphological features of mature cercariae and adults recovered from experimentally infected Wistar rats.

RESULTS

The stream was a shallow and permanent water body with moderately fast-flowing water and rocky bottom. Water temperature was 9.8 and 8°C, and pH varied from 5 to 6 in the first and second surveys, respectively. Snails were found attached to the underside of submerged stones and to aquatic vegetation composed of species of the Haloragaceae and Amaranthaceae.

Snails were identified as *G. truncatula* by comparisons with European specimens of confirmed identity and with illustrations previously published (Yahia, 1997; Samadi et al., 2000; Glöer, 2002). In addition, they were distinguished from lymnaeid species previously reported in Argentina (Table 1). Snails that were not used for taxonomic determination (n = 124) were

Table 1

Main morphological characters used to differentiate *Galba truncatula* from other lymnaeid species so far reported from Argentina. nd: no data available.

	G. truncatula	*L. viatrix*	*L. diaphana*	*L. columella*	*L. pictonica*
shell	less developed body whorl, with rounded shoulder; long spire			voluminous body whorl, with vaulted shoulder; short spire	
prostate	stomach-shaped, increases in diameter toward vas deferens	ovoid or pear-shaped	voluminous, irregular	thread-like or ribbon-like	nd
penial sheath/ prepuce ratio	1/4 to 1/5	1/1 to 1/3	1/1 to 1/2	1/6 to 1/8	nd
relative size of penial complex	++	+++	++++	+	nd
sources	Hubendick (1951) Godan (1979) Glöer (2002) Rondelaud (2007) present study	Paraense (1976) material collected by us	Paraense (1984) material collected by us	Paraense (1983) material collected by us	Hubendick (1951)

Table 2

Shell morphometrics (mean, standard deviation and min-max) of *Galba truncatula* from Sierras del Palauco, Province of Mendoza, Argentina. Measurements were made in 33 specimens.

	mean ± SD (mm)	min-max (mm)
Shell length	8.00 ± 0.78	6.00–9.50
Shell width	4.01 ± 0.39	3.16–4.90
Aperture length	3.90 ± 0.38	2.84–4.74
Aperture width	2.16 ± 0.27	1.42–2.69
Shell length/Shell width	2.00 ± 0.10	1.71–2.22
Shell length/Aperture width	2.05 ± 0.12	1.79–2.43

assumed to belong to *G. truncatula* because they were identical in external appearance to those already identified.

The length of all collected snails ranged between 1.74 and 10.27 mm. Shell measures obtained from 33 of the largest specimens are shown in Table 2. In the latter group, the penis sheath length to prepuce length ratio was 1:4 to 1:5. The shell and the most conspicuous organs of the male genitalia are shown in Figure 2 A–C.

Voucher specimens were deposited in the National Collection of Invertebrates at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia," Buenos Aires, Argentina (MACN-In, voucher number 37107).

Snail infection with *F. hepatica* was detected in the survey of June, with a prevalence of 2% (1/50). The infected snail measured 5.06 mm in shell length.

DISCUSSION

According to Heppleston (1972), *G. truncatula* reaches sexual maturity at a shell length of about 4.5 mm. On this basis, all snails used for species identification and the one infected with *F. hepatica* were adults. In the Old World, *G. truncatula* is the most important intermediate host of *F. hepatica* in Europe and some parts of Asia and Africa (Jabbour-Zahab et al., 1997). This snail is found in freshwater environments located at different altitudes, from lowlands at sea level to plateaus and mountain areas above 2000 m asl (Goumghar et al., 2001; Vignoles et al., 2002). It can colonize artificial and natural, permanent and temporary freshwater habitats (Heppleston, 1972; Hammami & Ayadi, 1999). This is probably accounted for by a remarkable ability to adapt to a wide range of water conditions including salinity and pH (Hammami & Ayadi, 1999). In addition, *G. truncatula* is able to withstand temperature and humidity fluctuations (Heppleston, 1972), drought through aestivation (Torgerson & Claxton, 1999), and very low temperatures through hibernation (Vareille-Morel et al., 1998). In Sierra del Palauco, snails were active at water temperatures of 8–9.8°C, in agreement with Heppleston (1972) who suggested a threshold value of 6°C.

In the Americas, the presence of *G. truncatula* has been ascertained in the Bolivian Altiplano by anatomical (Oviedo et al., 1995) and isoenzymatic studies (Jabbour-Zahab et al., 1997), as well as by 18S rDNA sequence analysis (Bargues et al., 1997). The results of these studies led authors to hypothesize that this species was introduced from Europe to Bolivia. Thus, the high ecological plasticity of *G. truncatula* may have enabled it to colonize successfully habitats under extreme conditions of very high altitude, between 3500 and 4200 m above sea level (asl) (Mas-Coma et al., 1999). This species has also been found in Valdivia, Chile (Yahia, 1997), a site located closer (39°48′00″S 73°13′59″W) to Sierra del Palauco, but with different

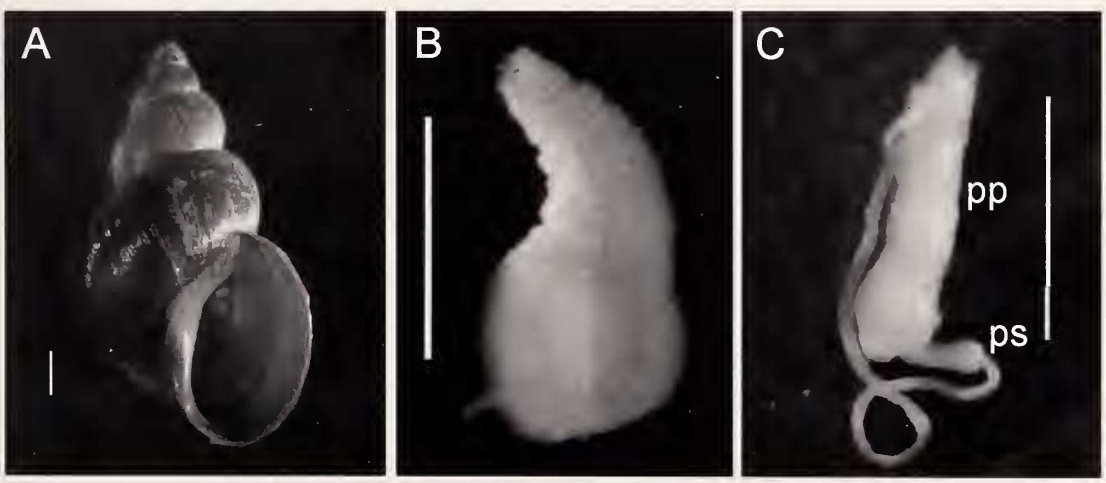

Figure 2. *Galba truncatula* from Sierras del Palauco, Province of Mendoza, Argentina. A: ventral view of shell; B: prostate; C: penial complex showing prepuce (pp) and penial sheath (ps). Scale bar = 1 mm.

environmental conditions (e.g., 5 m asl, temperate-rainy climate).

Although in Europe *G. truncatula* has been characterized as a mud, amphibious snail (Heppleston, 1972; Torgerson & Claxton, 1999), in Sierra del Palauco it was found attached to the underside of submerged stones. This fact suggests "a more aquatic" behavior, in accordance with that observed in snails of the northern Bolivian Altiplano (Mas-Coma, 1998).

In the endemic provinces of Argentine Patagonia, *L. viatrix* has been considered the only intermediate host involved in the transmission of *F. hepatica* (Kleiman et al., 2004, 2007; Rubel et al., 2005). However, the occurrence of infection in *G. truncatula* reported herein raises further questions regarding its importance in the local transmission cycle, because of the major role played by this species in Europe and the Bolivian Altiplano.

Acknowledgments. We are particularly indebted to Dr. Jean-Pierre Pointier at the Laboratoire de Biologie Marine et Malacologie, EPHE, Université de Perpignan, France, for providing the European *G. truncatula* specimens and for confirming the identity of our snails, to Dr. Patricia Hoc for the taxonomic determination of the aquatic vegetation, to Dr. Margarita Ostrowski for translating Glöer's text into Spanish, to Dr. Darío Vezzani for his help with the photographs, to the park rangers of Reserva Provincial La Payunia for their field assistance and hospitality, and to Dr. Peter M. Schantz for providing literature. Financial support was provided by Agencia Nacional de Promoción Científica y Tecnológica (BID 1728 OC-AR PICT 08830) and UBACYT (PID 2004–2007, X246)

LITERATURE CITED

Bargues, M. D., A. J. Mangold, C. Munoz-Antolli, J. P. Pointier & S. Mas-Coma. 1997. SSU rDNA characterization of lymnaeid snails transmitting human fascioliasis in South and Central America. Journal of Parasitology 83:1086–1092.

Cabrera, A. L. & A. Willink. 1980. Biogeografía de América Latina, Monografía 13, Serie Biología, Organización de los Estados Americanos, Washington. 120 pp.

Cucher, M. A., S. Carnevale, L. Prepelitchi, J. H. Labbé & C. Wisnivesky-Colli. 2006. PCR diagnosis of *Fasciola hepatica* in field-collected *Lymnaea columella* and *Lymnaea viatrix* snails. Veterinary Parasitology 137:74–82.

Esch, G. W., M. A. Barger & K. J. Fellis. 2002. The transmission of digenetic trematodes: style, elegance, complexity. Integrated and Comparative Biology 42: 304–312.

Glöer, P. 2002. Die Tierwelt Deutschlands. Die Süßwassergastropoden Nord- und Mitteleuropas Part 73. Conchbooks: Hackenheim. 327 pp.

Godan, D. 1979. Schadschnecken und ihre Bekämpfung. Eugen Ulmer (ed), Stuttgart. 97 pp.

Goumghar, M. D., P. Vignoles, D. Rondelaud, G. Dreyfuss & M. Benlemlih. 2001. Relations entre les générations annuelles de *Lymnaea truncatula* (Mollusca Gastropoda Lymnaeidae), l'altitude et la nature de ses habitats dans le centre du Maroc. Revue de Médecine Vétérinaire 152:457–462.

Hammami, H. & A. Ayadi. 1999. Écologie de *Lymnaea truncatula* Müller, hôte intermédiaire de *Fasciola hepatica* Linné dans le microclimat de Tozeur (Sud-ouest de la Tunisie). Bulletin de la Société de Pathologie Exotique 92: 302–304.

Heppleston, P. B. 1972. Life history and population fluctuations of *Lymnaea truncatula* (Müll.), the snail vector of fascioliasis. Journal of Applied Ecology 9:235–248.

Hubendick, B. 1951. Recent Lymnaeidae. Their variation, morphology, taxonomy, nomenclature and distribution. Kungliga Svenska Vetenskapsakademiens Handlingar, Fjarde Serien, Band 3. 223 pp.

Jabbour-Zahab, R., J. P. Pointier, J. Jourdane, P. Jarne, J. A. Oviedo, M. D. Bargues, S. Mas-Coma, R. Anglés, G. Perera, C. Balzan, K. Khallayoune & F. Renaud. 1997. Phylogeography and genetic divergence of some lymnaeid snails, intermediate hosts of human and animal fascioliasis, with special reference to lymnaeids from the Bolivian Altiplano. Acta Tropica 64: 191–203.

Kleiman, F., S. Pietrokovsky, W. L. Paraense & C. Wisnivesky-Colli. 2004. Southernmost finding of *Lymnaea viatrix* Orbigny, 1835 (Pulmonata: Lymnaeidae), intermediate host of *Fasciola hepatica* (Linnaeus, 1758) (Trematoda: Digenea), in urban and rural areas of Patagonia, Argentina. Memórias do Instituto Oswaldo Cruz 99:23–24.

Kleiman, F., S. Pietrokovsky, L. Prepelitchi, A. E. Carbajo & C. Wisnivesky-Colli. 2007. Dynamics of *Fasciola hepatica* transmission in the Andean Patagonian valleys, Argentina. Veterinary Parasitology 145:274–286.

Malek, E. A. 1985. Snail hosts of schistosomiasis and other snail-transmitted diseases in Tropical America: a Manual. PAHO: Washington. 325 pp.

Mas-Coma, S. 1998. Human fascioliasis in Europe and Latin America. Pp. 297–313 in M. Angelico (ed.), Infectious Diseases and Public Health. Balaban Publishers: Rehovot, Israel.

Mas-Coma, M. S., J. G. Esteban & M. D. Bargues. 1999. Epidemiology of human fascioliasis: a review and proposed new classification. Bulletin of the World Health Organization 77:340–346.

Oviedo, J. A., M. D. Bargues & S. Mas-Coma. 1995. Lymnaeid snails in the human fascioliasis high endemic zone of the northern Bolivian Altiplano. Research and Reviews in Parasitology 55:35–44.

Paraense, W. L. 1976. *Lymnaea viatrix*: a study of topotypic specimens (Mollusca: Lymnaeidae). Revista Brasileira de Biologia 36:419–428.

Paraense, W. L. 1982. *Lymnaea viatrix* and *Lymnaea columella* in the Neotropical region: a distributional outline. Memórias do Instituto Oswaldo Cruz 77:181–188.

Paraense, W. L. 1983. *Lymnaea columella* in northern Brazil. Memórias do Instituto Oswaldo Cruz 78:477–482.

Paraense, W. L. 1984. *Lymnaea diaphana*: a study of topotypic specimens (Pulmonata: Lymnaeidae). Memórias do Instituto Oswaldo Cruz 79:75–81.

Prepelitchi, L., F. Kleiman, S. M. Pietrokovsky, R. A. Moriena, O. Racioppi, J. Alvarez & C. Wisnivesky-Colli. 2003. First report of *Lymnaea columella* Say, 1817 (Pulmonata: Lymnaeidae) naturally infected with *Fasciola hepatica* (Linnaeus, 1758) Trematoda: Digenea) in Argentina. Memórias do Instituto Oswaldo Cruz 98:889–891.

Rondelaud, D. 2007. http://abela.club.fr/Articles/Limnees/Limnees_de_la_faune_de_France.html

Rubel, D., L. Prepelitchi, F. Kleiman, S. Carnevale & C. Wisnivesky-Colli. 2005. Estudio del foco en un caso de fasciolosis humana en Neuquén. Medicina 65:207–212.

Samadi, S., A. Roumegoux, M. D. Bargues, S. Mas-Coma, M. Yong & J. P. Pointier. 2000. Morphological studies of Lymnaeid snails from the human fascioliasis endemic zone of Bolivia. Journal of Molluscan Studies 66: 31–44.

Torgerson, P. & J. Claxton. 1999. Epidemiology and control. Pp. 113–149 in J. P. Dalton (ed.), Fasciolosis. CABI Publishing: London.

Vareille-Morel, C., M. Abrous, G. Dreyfuss & D. Roundelaud. 1998. Oviposition behaviour of *Lymnaea truncatula* in central France. Journal of Molluscan Studies 64:387–391.

Vignoles, P., L. Favennec, G. Dreyfuss & D. Rondelaud. 2002. Highland populations of *Lymnaea truncatula* infected with *Fasciola hepatica* survive longer under experimental conditions than lowland ones. Parasitology Research 88:386–388.

Yahia, H. 1997. Biosystématique de mollusques Lymnaeidae, hôtes intermédiaires de la fasciolose en Amérique Latine, DEA. Biologie de l'Evolution et Ecologie, Université Montpellier II, 39 pp.